An analysis of the seasonality of wood formation in cinnamon trees and eucalyptuses by knife-cutting method

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クスノキとユーカリにおける木部形成の季節性のナイフカッティング法による追跡

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Résumé

A knife-cutting method, tested by Fujiwara (1992), was re-examined on cinnamon trees and eucalyptuses which are generally growing in warm temperate zone or subtropical zone in order to investigate the possibility of cambial marking which is effective for the analysis of the seasonal characteristics of wood formation in tropical trees. As the wound tissue formed by knife insertion was limited in tangential width, it enabled application of several markings in a narrow area. The long slit-like wound tissue also had technical advantage in analyzing the characteristics of marking over the pinning method proposed by Wolter (1968). From the analysis of the wound tissues formed by monthly marking, especially "indirectly affected zone", or the zone with the tissue affected a little by mark ing, the position of cambial initials at the time of marking were more exactly estimated. The distance between the position and the annual ring boundary was measured as the radial increment. The seasonal characteristics of radial increment were discussed in relation to the climatic data.

要　　　旨

年輪構造の不明瞭な熱帯樹木における木部肥大成長リズムを詳細に追跡する形成層へのマーキング法を検討するために，Fujiwara (1992) が提案したナイフカッティング法を暖温帯に生育するクスノキとユーカリを用いて追試した。この方法ではWolter (1968) が提案した刺針法と同様に，傷害の影響が非常に狭い範囲に限られるので，樹幹の限られた部分に多くのマーキングを行うことが可能であり，また従来の刺針法に比べて縦方向に長く傷害が与えられるので，技術的な面でその後の解析が容易である。本研究では毎月1回マーキングを行って形成された「間接的影響領域」，つまり傷害の影響をわずかに含んでいる領域を詳細に観察することにより，まず，マーキング時の形成層始原細胞位置をより正確に推定した。次に，年輪界を測定の基準線として，その木部肥大成長リズムを月単位で追跡した。また，肥大成長リズムを気象データと比較して時期的対応を考察した。

1. Introduction

Although tropical deforestation has been a global problem in recent years, there is limited information on the seasonality of growth pattern in tropical trees. This is due to the fact that tropical trees have, in general, unclear annual ring boundaries.

For precise estimation of seasonal radial increment in tropical trees, it is effective and useful to mark each stage of xylem growth in tissue using a certain method. Mariaux[1] proposed a method called, "a window of Mariaux" in which bark of some centimeters square was removed as a time-marker. Some researchers, such as Sass et al.[2] applied the method to the analysis of wood formation in tropical trees. By this method, however, cambial zone receives too large injury to measure the radial increment in a limited area. It was therefore desirable to use a tiny injury as a time marker.

Pinning method which Wolter[3] invented improved the way of marking because the wound tissue formed after pin insertion was very small. Shimaji and Nagatsuka[4] applied it to Japanese fir. Yoshimura et al.[5], Yoshimura et al.[6] and Kuroda and Shimaji[7] contributed to get more precise information of the marking of cambial zone and differentiating zone mostly using conifers. Kuroda and Shimaji[8] also applied it to broad-leaved trees in temperate zone..

In the application of the pinning method to tropical trees, Shiokura[9] used a nail instead of a pin. This is because tropical trees have generally thick and hard bark. He used the term "nailing". The structure induced by nailing was basically similar to that formed by pin insertion. He pursued the radial increment in relation to seasons. The information on exact marking of cambial zone and differentiating zone, however, was not reported in his study. In their minute observation of the wound tissue formed by nailing, Nobuchi et al.[10] estimated the position of cambial initials at the time of marking in tropical trees.

It was considered that wound tissue formed by pin insertion were short in longitudinal direction where the exact information of marking were recorded. A "knife-cutting method " proposed by Fujiwara[11] was the alternative method to improve the pinning method. It was expected not only to give similar anatomical features as those of the pinning method but also to give an technical convenience of having slit-like wound tissue where the exact information was recorded.

Although Jalil et al.[12], Ogata et al.[13] and Kondo et al.[14] prosecuted knife-cutting method, information on any differences between knife-cutting and pinning method, however, remain unresolved.

In this report, therefore, the methodology of knife-cutting was investigated in comparison with the pinning (nailing) method. After clarifying the methodology, the seasonality of radial increment in trees of temperate zone was analyzed as a fundamental study for the application to tropical trees.

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2. Materials and methods

2. 1 *Sample trees*

Two cinnamon trees (*Cinnamomum camphora*) and three eucalyptuses (two *Eucalyptus tereticornis* and one *E. saligna*, Fig. 1) were used for this study. Description of the sample trees is given in Table 1.

Table 1 The description of sample trees

Tree name	Species	Height (m)	DBH (cm)	Tree Age	Date of felling
cinnamon tree No.1	*Cinnamomum camphora*	4.2	8.7	6	Dec.8,1994
〃 No.2	〃	7.1	18.0	8	Dec.16,1994
eucalyptus No.1	Eucalyptus tereticornis	8.0	8.4	3	May.9,1996
〃 No.2	〃	10.1	11.3	4	Dec.8,1994
〃 No.3	Eucalyptus saligna	12.8	11.6	4	May.9,1996

2. 2 *Cambial marking and collection of wood blocks*

To compare the characteristics of the two types of wound tissues inflicted by nailing (pinning by a nail) and knife-cutting, two series of marking were carried out in two cinnamon trees and two eucalyptuses (*E. tereticornis*). A nail of 2 mm in diameter and a knife of 0.5 mm in thickness and 10 mm in height were used, with intervals of 3-4 cm between two adjacent marking points. The nail and the knife were removed immediately after marking. The distance between two horizontal lines was about 10 cm. To speculate the characteristics of the wound tissues formed by the different sizes of tool, a pin of 0.5 mm in diameter and a special knife of 2 mm in thickness and 15 mm in height were also used in August 1995.

Marking was done basically every month from April 1994. Table 2 indicates the dates of marking. Sample trees were felled in December 1995 except eucalyptus No. 1, which was felled in May 1996.

Only knife-cutting was applied to eucalyptus No. 3 every month from May to November 1995 and it was felled in May 1996.

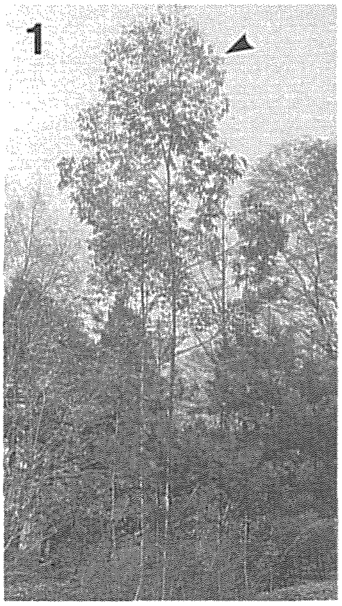
To investigate the cambial activities at the time of marking, wood blocks which were not affected by nail or knife insertion were also collected from a cinnamon No. 2 and eucalyptus No. 2 in June, September and December 1994. These blocks were considered to represent directly the actual structure of cambial zone and differentiating zone.

Table 2 Dates of marking

1994*	Apr.26	1995**	Apr.17
	May.31		May.22
	Jun.28		Jul.28
	Aug.5		Aug.21
	Aug.31		Sep.19
	Sep.30		Oct.17
	Oct.31		Nov.16
	Dec.1	1996**	Apr.18

* Dates of marking in cinnamon trees and eucalyptus No.1 and No.2

**Dates of marking in eucalyptus No.1 and No.3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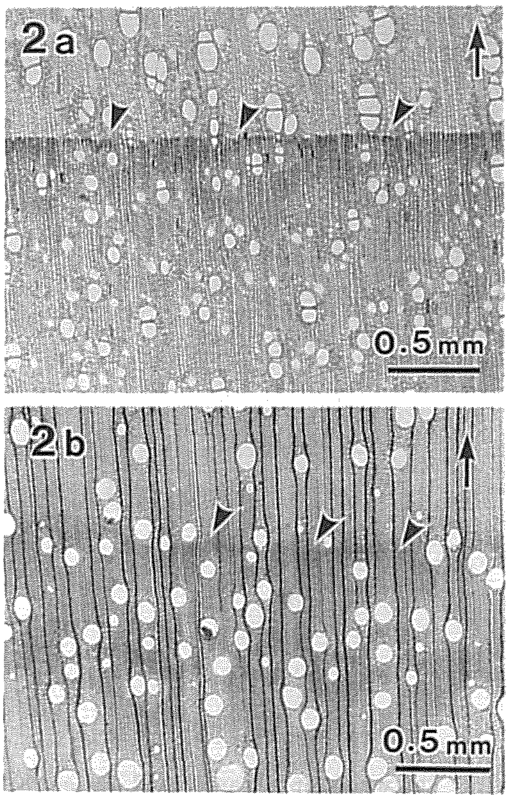
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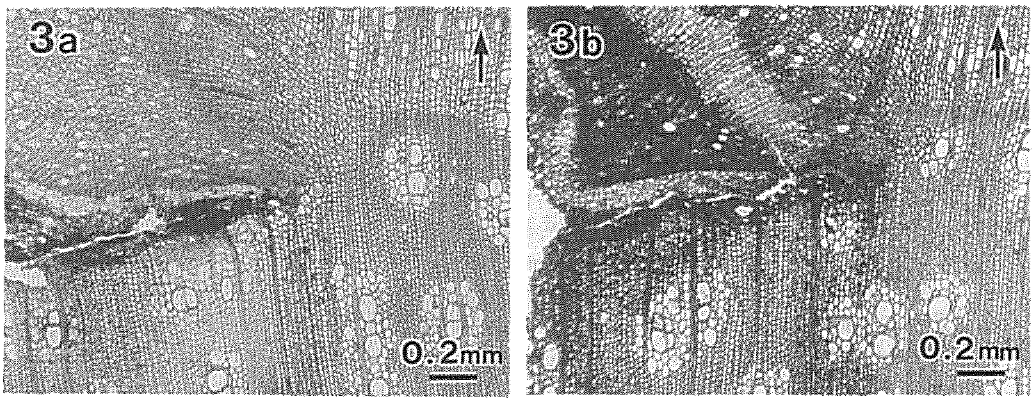
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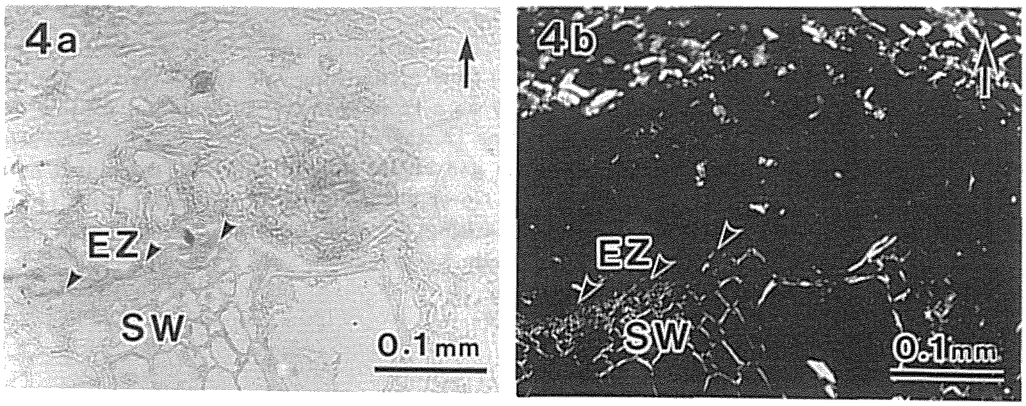
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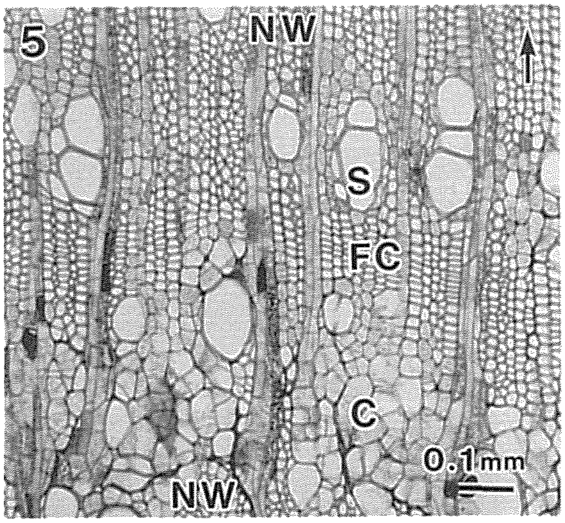

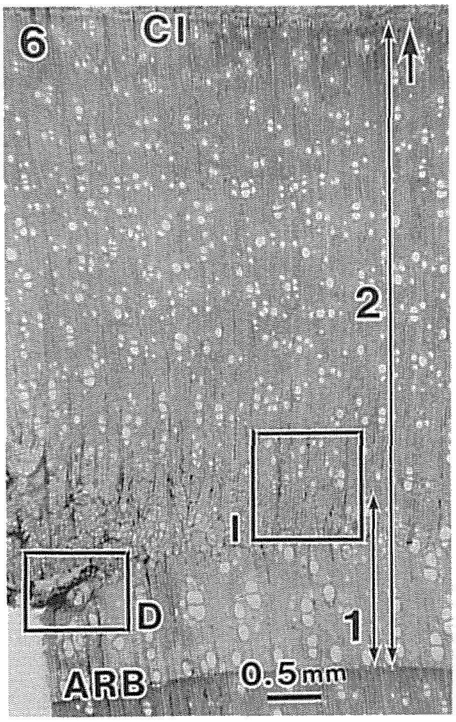

Fig. 1 The whole appearance of *E. saligna* (arrowhead)

Fig. 2 Transverse sections showing reference line, or annual ring boundaries of cinnamon tree No.2 (a), and eucalyptus No.2 (b). Arrowheads represent the reference lines. Arrows in Fig.2-6 indicate the direction of bark side.

Fig. 3 Transverse sections of cinnamon tree No.2 showing wound tissues by nailing (a) and by knife-cutting (b).

Fig. 4 Transverse sections of cinnamon tree No.2 marked in September 1994 showing directly influenced zone corresponding to zone D in Fig.6 observed under a ordinary (a) and a polarizing microscope (b). EZ, SW indicate enlarging zone and secondary wall thickening zone at time of marking, respectively.

Fig. 5 Transverse section of indirectly influenced zone, corresponding to zone I in Fig.6. NW: normal wood formed before marking; C: callus-like cells corresponding to enlarging zone at the time of marking as deduced in directly influenced zone; FC: radially flat cells showing undifferentiated cells affected by marking; S: the layer including small vessel elements formed after marking.

Fig. 6 A transverse section of cinnamon tree No.2. Line 1 and line 2 indicate radial increment from the beginning of growth to the time of marking and to the time of felling, respectively. ARB: annual ring boundary as a reference line; CI: cambial initials at the time of felling. Radial increment (line 1) was measured as the distance between ARB and deduced site of cambial initials and radial growth rate was calculated as the percentage of radial increment (line 1) over the given width of annual ring (line 2). Zone D: tissue directly affected by marking. Zone I: indirectly affected tissue not crushed but showed abnormal differentiation by marking.

The girth at a fixed height (1.3 m from the ground level) of all sample trees was also measured with a tape measure on the same day of marking.

2. 3 *Light microscopy*

Wood blocks were fixed with 3% glutaraldehyde immediately after collection. After a few days the fixative was changed to 30% Et-OH in room temperature for preservation of the wood tissues.

Transverse sections, 30μm in thickness, were cut using a sliding microtome. They were stained with safranin and fast green. Wound tissues together with the general structure were observed under an ordinary microscope.

Small wood blocks which were not affected by marking were embedded in Epon 812. Sections, 3μm in thickness, were cut with a glass knife on a rotary microtome (Type JB-4) and stained with safranin. These thin sections were observed under a polarizing microscope.

3. Results and discussion

3. 1 *Anatomical characteristics of annual ring boundary as a reference line*

The characteristics of annual ring boundary as a reference line were investigated in transverse sections, because the concentric line is necessary for measurement of radial increment at different marking positions in each tree.

Cinnamon trees (Fig. 2a) had distinct annual ring boundaries in alteration of radial diameter of vessels and of wall thickness and radial diameter of wood fibers, which were useful as reference lines.

Both species of eucalyptuses (Fig. 2b, representing only *E. tereticornis*) had unclear annual rings because of little variation in diameter of the vessels observed throughout one year. Detailed observation, however, revealed that late wood had slightly smaller diameter of vessels and thicker walls of fibers than early wood. Judging from these characteristics, eucalyptuses also had the discernible reference lines.

3. 2 *Comparison between the characteristics of knife-cutting and nailing*

The characteristics of two types of marking - knife-cutting and nailing - were compared (Fig. 3). In general, wound tissues formed by knife or nail insertion showed similar patterns. Additionally, each type of marking gave analogous characteristics in wound tissue despite of the difference in the range of the tissues between two different-sized tools. In nail insertion, however, the range of wound tissue in longitudinal direction in which the exact marking of cambial zone could be investigated was limited in directly affected zone, as mentioned below. Therefore, knife-cutting was technically convenient for preparation because it had wider range of directly affected zone in longitudinal direction over nail insertion.

3. 3 *Estimation of the position of cambial initials at the time of marking*

A transverse section of a wound tissue induced by knife-cutting is shown in Fig. 6. Two zones in the wound tissue were focused in this report. In zone D in Fig. 6, cells in cambial and enlarging zone had been directly affected by knife-cutting and crushed. Cells in this zone, therefore, ceased their maturing at the time of knife cutting. In zone I, cells in cambial and enlarging zone were not crushed but affected by knife-cutting resulting in the formation of abnormal cells, as mentioned later. In this paper, the former is termed "directly affected zone" and the latter "indirectly affected zone".

Directly affected zone

A sample of directly affected zone was observed under a polarizing microscope (Fig. 4). Under cross-Nicol, only the crushed cells showed no birefringence. The crushed cells, therefore, were considered to be the cells of cambial and enlarging zone (EZ). The boundary between crushed and non-crushed cells (arrowheads) was judged to be the boundary between the enlarging zone and secondary wall thickening zone (SW).

Indirectly affected zone

Anatomical characteristics of indirectly affected zone showed a region of normal wood (NW), callus-like cells (C), radially flat cells (FC), a layer with small diameter vessel elements (S) and normal structure of wood from the pith side to the bark side (Fig. 5), though callus-like cells are somewhat close to the site of marking.

The callus-like cells, which corresponded to the cells of enlarging zone in directly affected zone, were considered to have been formed by ray parenchyma cells to fill the gap formed after knife insertion. This layer included the residues of differentiating and enlarging zones at the time of marking which corresponded to "the stripe of cell wall residue" reported by Yoshimura et al. (1981).

The group of radially flat cells were considered to have been the cambial zone at the time of marking which remained undifferentiated after marking and retained structural characteristics of cambial zone though it was not clear that they included all of the cells in cambial zone.

It was considered that the small diameter vessels abnormally differentiated from cambial cells affected by knife cutting which represented that this region was formed after marking.

From the features mentioned above, it was assumed that the cells on the boundary between the layer of radially flat cells and the layer with small diameter of vessels are the cells reactivated after marking. In this report, it was proposed that these cells is the cambial initials at the time of marking.

Fujiwara (1992) estimated the position of cambial zone and enlarging zone at the time of marking only in directly affected zone. The cells of directly affected zone, however, were generally crushed by knife insertion as mentioned above. It is, therefore, difficult to deduce the exact position of cambial initials at the time of marking. In this study we estimated the cambial initials more precisely in the analysis of the indirectly affected zone, together with the directly affected one.

3．4 *Seasonality of radial growth rate*

The distance between the reference line and the estimated cambial initials at the time of each marking was measured (line 1 in Fig. 6). This indicates the radial increment in the term from the beginning of xylem formation in spring to the time of a given marking. The radial increments in a year, in millimeter are shown in Fig. 7a.

It is, however, quite natural that some errors might arise because the radial increment is not homogenous along the circumference of the trunk. To avoid such errors, the annual increment was expressed as percentage of radial distance between the reference line and the cambial initials at the time of felling (line 2 in Fig. 6).

The percentage data for each radial increment in the year are shown in Fig. 7b. The radial growth in cinnamon tree No. 2, showed active increase from April to July 1994, became slow in August and again showed active increase in September and October until the cessation of growth in November. The relationship between radial increment and climatic data is discussed in the later part of the paper.

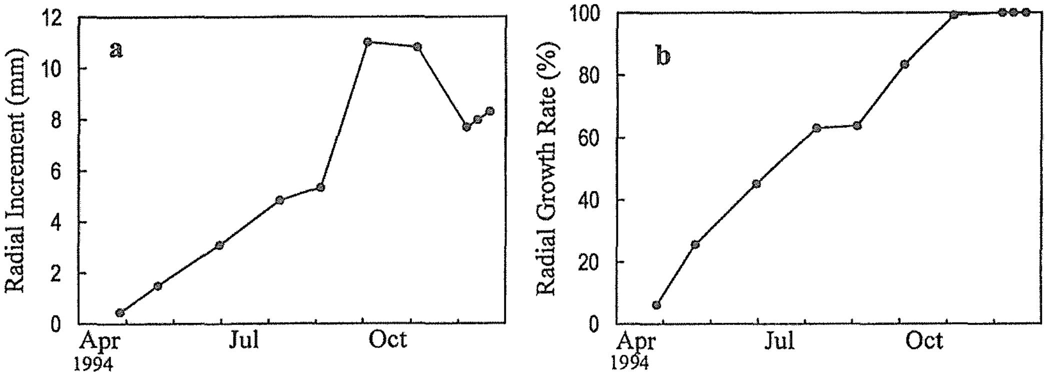

Fig. 7 The seasonal change of radial increment (a) and radial growth rate (b) in cinnamon tree No.2

3．5 *The measurement of radial increment with a tape measure*

The girth of a trunk at the breast height was measured with a tape measure on the day of marking and the results including the radial increment are shown in Fig. 8. Indirect measurement of xylem radial increment showed similar tendency as the direct measurement by marking in this experiment. Girth measurement is a convenient method to estimate and pursue the radial

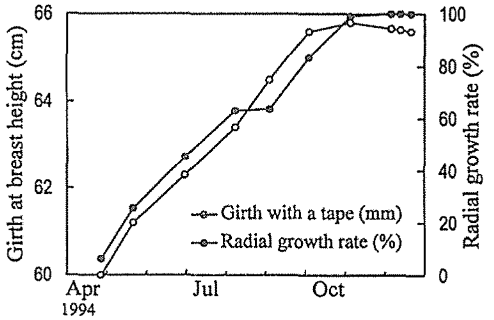

Fig. 8 Comparison of the girth measured with a tape measure to radial growth rate of cinnamon tree No.2

increment of a tree. It might, however, have some errors caused by the removal of bark, daily fluctuation caused by water stress, uneven or irregular surface condition of a trunk, etc. Because of these factors, the girth measurement does not always give exact results of radial increment.

3. 6 *Estimation of Seasonality of cambial activity*

From cinnamon tree No. 2 and eucalyptus No. 2, wood blocks containing cambial zone and differentiating zone in the region having no effect of marking were collected and investigated in June, September and December 1994. Total cell numbers of both cambial zone and enlarging zone were counted under a polarizing microscope (Table 3). The number of cells was considered to reveal the cambial activity at the time of sample collection. In both species, cambial zone showed high activity in June and September, and cinnamon No. 2 showed dormancy in December while the eucalyptus still showed cambial activity though the radial growth rate showed dull increase (Fig. 10).

Table 3 Number of cells of cambial zone and enlarging zone

	cinnamon tree No.2			eucalyptus No.2		
	CZ	EZ	CZ+EZ	CZ	EZ	CZ+EZ
1994 Jun.28	8	12	20	11	11	22
Sep.30	9	13	22	11	12	23
Dec.1	6	0	6	12	17	29

Note: CZ and EZ show the number of the cells of cambial zone and enlarging zone, respectivily.

3. 7 *Relationships among xylem radial growth, cambial activity and climatic factors*

To discuss the relationships among radial increment, cambial activity and climatic factors, the percentage data of radial increment of each tree species were compared with climatic data together with the measured cambial activity (Figs. 9, 10).

In cinnamon trees (Fig. 9), radial increment showed good coincidence with increase of temperature from April to July 1994. However, in July to August when the rainfall was much less than usual, the increase of radial growth was reasonably low even though the temperature was high enough. In September, the growth recovered and gradually got lower until its cessation in November parallel with the decrease of the temperature. The amount of radial growth,

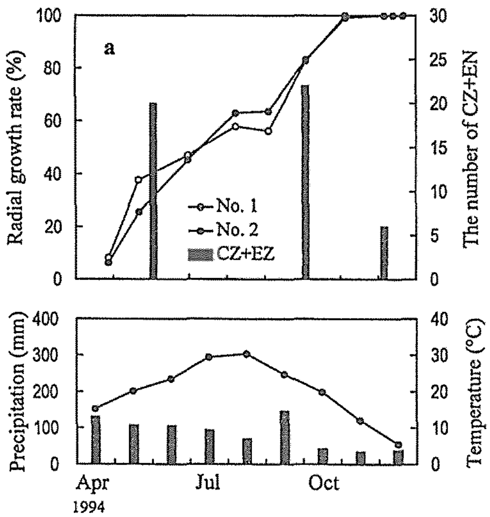

Fig. 9 Relationships among radial growth, cambial activity and climatic data for cinnamon trees.

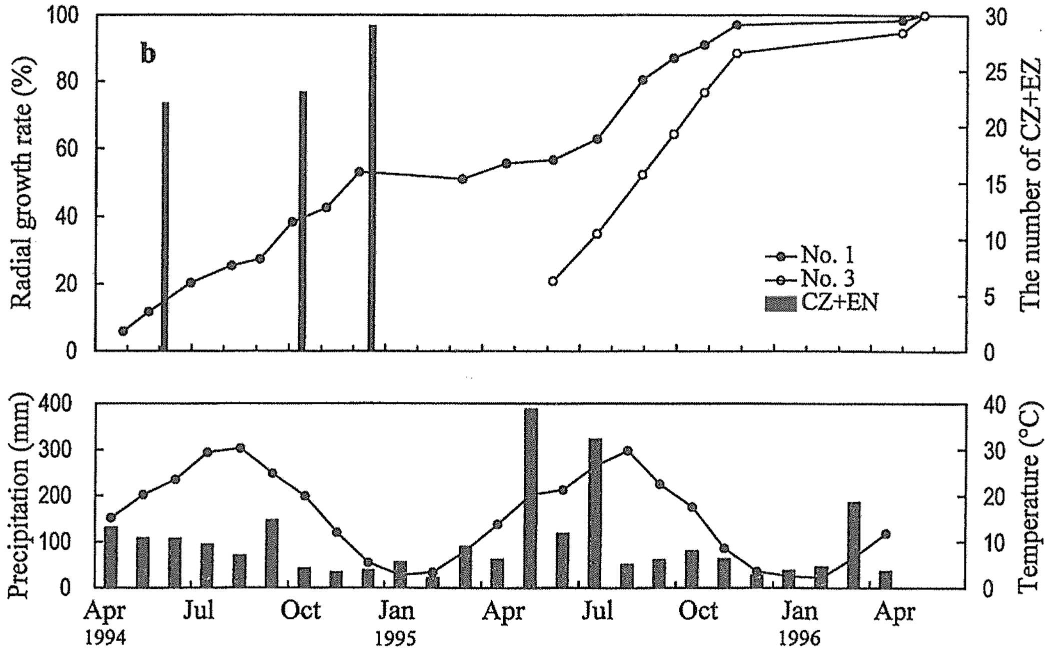

Fig. 10 Relationships among radial growth, cambial activity and climatic data for eucalyptuses.

moreover, showed good coincidence with the cambial activity, i.e. it was high in June and September, when the cambial activity was also active. Little radial growth was observed in November when the cambial zone showed a pattern of dormancy. In cinnamon, the temperature is considered to be the dominant factor that controls cambial activity and radial increment. Another important point was that little amount of rainfall also arrested the cambial activity even if the temperature was high enough for its activity.

Eucalyptuses (Fig. 10) showed basically similar tendency as the cinnamon trees. In November 1994, however, it continued radial growth unlike the cinnamon trees though the amount of radial growth was less. In July and August 1994, eucalyptus No. 1 formed false annual rings, while no false annual ring in 1995. This difference revealed that the lower precipitation in the summer of 1994 affected the cambial activity resulting in the formation of false annual ring. One of the reasons for the differences among these species might be endogenous factors. Additionally, there were a large number of cells in cambial and enlarging zones in December 1994 in spite of little radial increment during the month. Further investigation is necessary to clarify the relationships among the radial growth, cambial activity and climatic factors.

3. 8 *Efficiency of knife-cutting method as a time marker*

From the investigation presented above, it was revealed that knife-cutting is a useful and efficient tool as a time marker. It is expected to be applied even to tropical trees without annual rings as long as they have some concentric anatomical structures. The application of this method to tropical trees might contribute to studies both in ecology and silvicultural practices.

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